PATENT
034827-3002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Qu, K. et al.
Title:	COMPOSITIONS AND METHODS FOR DETERMINING GENOTYPES
Appl. No.:	10/714,508
Filing Date:	November 14, 2003
Examiner:	Mummert, Stephanie K.
Art Unit	1637
Confirmation Number	9832

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APPEAL BRIEF

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Sir:

Applicants (hereinafter "Appellants") hereby appeal the final rejection of claims 2 and 40-72, which correspond to all claims pending in the application. This Appeal Brief follows a Notice of Appeal filed February 8, 2007. The fee for this Appeal Brief (37 C.F.R. § 41.20(b)(2)) accompanies this filing. If the fee is absent or incorrect or if any additional fees are due in this regard, please charge or credit our Deposit Account No. 50-0872 for the appropriate amount.

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CASES

RULES

Real Party in Interest

The real party in interest in this appeal is Quest Diagnostics Investments Incorporated, the assignee of record.

Related Appeals and Interferences

None.

Status of Claims

Claims 1 and 3-39 have been cancelled.

Claims 2 and 40-72 are pending in the application.

Claims 2 and 40-72 are the subject of this appeal.

Status of Amendments

The last claim amendments were presented in Appellants' response of June 23, 2006. The claim amendments contained therein have been entered, examined, and are appealed herein. No further amendments or submissions are pending in the application.

Summary of Claimed Subject Matter

The human angiotensin converting enzyme (ACE) plays a central role in regulating blood pressure and electrolyte balance.[1] ACE has been identified as an important therapeutic target for diseases including hypertension, diabetic neuropathy, renal disease, and various cardiac disorders including, for example, congestive heart failure and myocardial infarction.[2]

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[1] Specification at ¶ 3.

[2] Id.

The human ACE gene is known to have two allelic variants based on the presence or absence of a 287 base pair non-coding fragment contained within Intron 16.[3] Individuals who are heterozygous or homozygous for the deletion ("D allele") have elevated ACE activity relative to those who are homozygous for the wildtype allele which contains the fragment ("I allele").[4] Presence of the D allele and the concomitant increase in ACE activity has also been associated with increased risk of certain heart diseases and an altered responsiveness to medically-important ACE inhibitors.[5] Thus, there is a need for rapid and reliable methods for determining the ACE genotype of an individual, with respect to Intron 16, in order to identify individuals having an elevated risk of developing cardiovascular disease and to deliver appropriate pharmaceutical therapy.

The claimed invention provides a single-step polymerase chain reaction (PCR) method for ACE genotyping. The method is based on the use of a pair of flanking nucleic acid primers that are capable of amplifying the entire genomic region of interest (i.e., flanking the insertion site), in combination with a third nucleic acid primer that specifically binds to the inserted fragment (i.e., the 287 based pair fragment of Intron 16) which causes the allelic variation. This primer strategy results in a different number of amplification products ("amplicons") from each possible genotype. Specifically, a single amplicon is produced from the D/D genotype; two different amplicons are produced from the I/I genotype; and all three amplicons are produced from the I/D genotype.[6] Thus, an individual's ACE genotype may be simply and rapidly determined based solely on the number of amplicons observed. The claimed method is a significant improvement over the prior art because it is a rapid and user-friendly assay with a simple diagnostic readout.

Claim 2, which is the first independent claim, relates to a method for determining the ACE genotype in a sample by amplifying the DNA using a pair of flanking primers and a third primer specific for the variant gene sequence, and then determining the ACE genotype based on

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[3] Id. at ¶ 5.

[4] Id. at ¶ 6.

[5] Id.

[6] Id. at Figure 1.

the number of amplification products. Support for claim 2 is found in the specification at ¶¶ 11, 13, 30, 55, and Figure 1.

Claims 49 and 54 depend ultimately from claim 2. Support for claims 49 and 54 is found in the specification at ¶¶ 31, and 43-44.

Claims 51, 52 and 60 depend ultimately from claim 2. Support for claims 51, 52 and 60 is found in the specification at ¶¶ 30 and 55.

Claim 62 relates to a method for identifying patients with a heightened risk of suffering from a cardiovascular disease by amplifying a DNA sample using a pair of flanking primers and a third primer specific for the variant gene sequence, and then determining the ACE genotype based on the number of amplification products, and correlating that genotype with a treatment regimen. Support for claim 62 is found in the specification at ¶¶ 11, 13, 30, 36-38 and 58-59, and Figure 1.

Claim 66 depends from claim 62. Support for claim 66 is found in the specification at ¶¶ 31, and 43-44.

Claim 67 relates to a method for determining a genotype of interest by amplifying DNA using a pair of primers that flank the region of interest and a third primer specific for the variant insertion sequence, and then determining the genotype based on the number of amplification products. Support for claim 67 is found in the specification at ¶¶ 10, 12 and Figure 1.

Grounds for Rejection to be Reviewed on Appeal

1. Claims 2, 40-48, 50 and 67-72 stand finally rejected under 35 U.S.C. § 103(a), as allegedly being obvious over Lindpaintner et al. (N. Engl. J. Med., 332: 706-711, 1995; "Lindpaintner") in view of Lin et al. (Clin. Biochem., 34: 661-666, 2001; "Lin").

2. Claims 49 and 51-61 stand finally rejected under 35 U.S.C. § 103(a), as allegedly being obvious over Lindpaintner in view of Lin, Soubrier et al. (U.S. Patent 5,736,323; "Soubrier"), and Buck et al. (Biotechniques, 27: 528-536; 1999; "Buck").

3. Claims 62-65 stand finally rejected under 35 U.S.C. § 103(a), as allegedly being obvious over Lindpaintner in view of Lin and van Bockxmeer et al. (Circulation, 92: 2066-2071, 1995; "van Bockxmeer").

4. Claim 66 stands finally rejected under 35 U.S.C. § 103(a), as allegedly being obvious over Lindpaintner in view of Lin, van Bockxmeer, Soubrier, and Buck.

5. Claims 2, 40-48, 50 and 67-70 stand finally rejected under 35 U.S.C. § 103(a), as allegedly being obvious over Teranishi et al. (J. Hypertens., 17: 351-356, 1999); "Teranishi") in view of Lin.

Argument

1. Appellants' Claimed Invention.

The human ACE gene is known to have two allelic variants at Intron 16 based on the presence or absence of a 287 base pair non-coding fragment.[7] The allele which contains the non-coding fragment is referred to as the "I allele", and the allele with the deletion is referred to as the "D allele". These two alleles give rise to three possible genotypes: I/I, I/D, and D/D.

Appellants' claimed invention is a method for determining ACE genotype, or the genotype of any allele known to have a deletion variant, using a single PCR amplification. The claimed method is based on the use of three nucleic acid primers which consist of a flanking primer pair and a single fragment-specific primer.[8] The flanking primer pair is selected to amplify the entire genomic region of interest, whether or not the fragment is present or deleted. Thus, amplification from the flanking primer pair will result in a single amplicon from either allele, but the D allele will produce an amplicon that is 287 nucleotides shorter than the amplicon produced from the I allele.[9]

The fragment-specific primer is designed to form a primer pair with one of the flanking primers. The fragment-specific primer will only hybridize with, and amplify the I allele which contains the fragment. Appellants disclose an exemplary fragment-specific primer which results in an amplicon from the I-allele which is a characteristically different length than either amplicon

[7] Id. at ¶ 5.

[8] Id. at ¶¶ 10 and 14.

[9] Id. at Figure 1.

produced by the flanking primers. Obviously, no amplicon is produced from the D-allele using this primer.[10]

Following amplification using Appellants' three-primer system, the genotype of an individual may be determined by merely counting the number of amplicons produced. Amplification of the D/D genotype results in only one (short) amplicon produced from the flanking primers. Amplification of the I/I genotype results in two amplicons: one from the flanking primers and a second from the fragment-specific primer. Finally, amplification of the I/D genotype results in three amplicons: the one amplicon from the D-allele and the two amplicons from the I-allele. Thus, identification of an individual's genotype may be determined using a single PCR reaction and merely counting the number of amplification products.[11]

2. The legal standard for obviousness.

In order to make a *prima facie* case of obviousness, the Examiner must demonstrate that the prior art (i) teaches or suggests every claim limitation, (ii) provides a motivation to combine (or modify) the teachings of the selected references, and (iii) provides a reasonable expectation of success. In re Vaeck, 947 F.2d 488, 493, 20 USPQ2d 1438 (CAFC 1991); MPEP § 2143. This is the "TSM" test for obviousness which was recently affirmed by the Supreme Court. KSR Int'l Co. v. Teleflex Inc., No. 04-1350, 550 U.S. ____, slip op. at 15 (2007). In explicating the correct standard for this test, the KSR Court reaffirmed previous holdings that an invention "is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." KSR, slip op. at 14.; see also, In re Rouffet, 149 F.3d 1350, 1357, 47 USPQ2d 1453, 1457 (Fed. Cir. 1998). Furthermore, the Court warned the factfinder to be aware of the distortion caused by hindsight bias and to be cautious of arguments reliant upon *ex post* reasoning. KSR, slip op. at 17.

3. Rejection of claims 2, 40-48, 50 and 67-72 under 35 U.S.C. § 103(a) as obvious over Lindpaintner in view of Lin.

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[10] Id. at ¶ 14.

Appellants respectfully traverse the rejection of claims 2, 40-48, 50 and 67-72 as allegedly being obvious over Lindpaintner et al. (N. Engl. J. Med., 332: 706-711, 1995; "Lindpaintner") in view of Lin et al. (Clin. Biochem., 34: 661-666, 2001; "Lin").[12] Appellants respectfully request that the rejection be reversed and withdrawn.

Appellants note that claim 67 encompasses a method for determining the genotype of a gene of interest which has allelic variants based on the presence or absence of an inserted sequence. Claim 2 encompasses a specific variant of the method of claim 67 in which the genotype of ACE is determined. For clarity, the following argument refers specifically to the ACE gene; however, the arguments apply equally to generic claim 67.

3.1 The obviousness rejection applied by the Examiner is wholly deficient.

The Examiner asserts a rejection based on the combined teachings of Lindpaintner and Lin. As discussed in more detail below, this combination fails to support an obviousness rejection because it does not teach every element of Appellants' claimed method. The Examiner, unable to identify each element of the claimed method in the prior art, attempts to obfuscate the deficiency.

Logic dictates that an obviousness analysis begins with the closest prior art and looks to other teachings to provide the missing elements and the motivation to combine those teachings. It is ridiculous to start with a secondary reference and attempt to supplement its teachings with a more relevant teaching. Such an analysis is analytically unsound and unnecessarily complicated. However, this is exactly what the Examiner has done.

The Examiner applies Lindpaintner as the primary reference when, in fact, Lin is the closest prior art. Lin performs a single PCR amplification using the same three primer strategy as Appellants. However, Lin's implementation of this three primer strategy, and its result, is irreconcilably different from Appellants implementation embodied by the rejected claims. This deficiency is not remedied by the Lindpaintner method which utilizes an entirely different genotyping strategy. Thus, the Examiner failed to articulate a well-reasoned obviousness

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[11] Id. at ¶ 55 and Figure 1.

[12] Final Office Action mailed September 11, 2006 at p. 3-6.

rejection which meets the minimum criterion of identifying in the prior art each element of Appellants' claimed method.

3.2 The prior art cited by the Examiner.

Lin

Lin provides a "real-time" PCR-based method for determining the ACE genotype at Intron 16. Lin performs a PCR amplification reaction using a three-primer system consisting of a flanking primer pair (ACE1 and ACE3) and an insertion fragment-specific primer (ACE 2). [13] Lin reports detecting a single 84 bp amplicon from the D-allele (derived from the ACE1-ACE3 primer combination) and a single 65 bp amplicon from the I-allele (derived from the ACE2-ACE3 primer combination). [14]

The amplicons are detected using melting curve analysis which measures the dissociation of duplexed DNA into individual strands, wherein the melting curve peak is dependent upon amplicon length.[15] Specifically, the 65 bp amplicon derived from the I-allele has a lower melting point than the 84 bp amplicon from the D-allele.

In sum, Lin performs a genotype determination using a single PCR amplification based on a three-primer combination consisting of a pair of flanking primers and a fragment-specific primer. Lin's implementation of this system results in one single amplicon from the I-allele and one single amplicon from the D-allele. The ACE genotype is determined by detecting the amplified fragments using a melting curve analysis. Thus, the homozygous I/I and D/D genotypes are each identified by a single amplicon which differs in size. The heterozygous genotype I/D is identified by the presence of both amplicons. In no case is a third amplicon identified.

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[13] Lin at Section 2.4 and Figure 1.

[14] Page 662, Section 2.4 notes that the primers were the same as described by Evans et al. Page 663, right column notes that Evans et al. reported fragments of 65 bp and 84 bp for the I- and D-alleles respectively. These fragment lengths are consistent with Figure 1.

[15] Figure 2, 3A, 4 and 5A.

Lindpaintner

Lindpaintner described the traditional two-step, four-primer method for determining ACE genotype. In the first step, genomic DNA is first amplified using a flanking primer pair (hace3s and hace 3as) in order to amplify the entire sequence of interest. The result is a long and a short amplicon corresponding to the I- and the D-alleles, respectively.[16] This PCR product is initially screened to identify ACE genotype, wherein the presence of only the long amplicon indicates the I/I genotype and presence of both amplicons indicates the I/D genotype.

Lindpaintner notes however that the shorter D-allele amplicon is preferentially amplified relative to the I-allele amplicon. Thus, samples lacking the I-allele amplicon must be further tested to confirm that its absence is truly indicative of a D/D genotype and not an artifact of differential amplification. Confirmation is done using a second PCR with a different primer pair (hace5a and hace5c) in which one member binds specifically to the fragment sequence.[17] The I-allele, which contains the fragment, gives rise to an amplification product; whereas, the D-allele, which lacks the fragment (i.e., lacks one primer binding site), does not yield an amplification product. Based on this secondary analysis, the samples lacking the I-amplicon from the first PCR are categorized as the I/D genotype (presence of amplicon) or the D/D genotype (absence of amplicon).

Genotype identification using the Lindpaintner method is as follows: The I/I genotype is identified by one single amplicon in the first PCR (the second PCR being unnecessary). The D/D genotype is identified by one single amplicon in the first PCR and the absence of an amplicon in the second PCR. The I/D genotype is identified either by the presence of two amplicons in the first PCR (the second PCR being unnecessary) or by the presence of the D-amplicon in the first PCR and the I-amplicon in the second PCR. This genotype criteria is summarized in the following table.

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[16] Lindpaintner at p. 707, right column, second paragraph; amplicons of 319-bp and 597-bp result from the D-allele and the I-allele, respectively.

[17] Lindpaintner at p. 707, right column, third paragraph.

Genotype	# of PCR Reactions	# of different primers used	# of amplicons detected (description of amplicons)
I/I	1	2	1 (long amplicon only in 1st PCR; second PCR not performed)
D/D	2	4	1 (short amplicon only in 1st PCR; no amplicon detected in 2nd PCR)
I/D	1	2	2 (long and short amplicon in 1st PCR second PCR not performed)
I/D (alternative)	2	4	2 (long amplicon only in first PCR; single amplicon detected in 2nd PCR)

3.3 The combination of Lin and Lindpaintner does not provide every element of Appellants' claimed method.

Lin provides a method for determining ACE genotype using a single PCR reaction and a three primer system. Lin, like Appellants, use a flanking primer pair and an internal, fragment-specific primer. However, the implementation and the result of this primer strategy is vastly different.

As an initial matter, claim 2 requires that a homozygous genotype is detected by the production of one or two amplification products and a heterozygous genotype by three amplification products. Using the claimed method, the D/D genotype is detected by a single product, the I/I genotype by two products, and the I/D genotype by three products. The Lin method, by contrast, produces one product from both the I/I and D/D genotypes and two products from the I/D genotype. Despite the similarities in primer design, the difference in product number is a function of implementation.

Appellants' method produces two amplicons from the I-allele; whereas Lin produces only one single amplicon. Specifically, Appellants produce one amplicon from the flanking primers (ACE1 and ACE 3 of Lin) and another amplicon from the fragment-specific primer and one flanking primer (ACE2 and ACE3 of Lin). By contrast, Lin produces only the latter. This difference is specifically demonstrated in Figure 2 of Lin which shows a gel electrophoresis of

Lin's amplification products. Clearly visible is the single amplicon from the D-allele ("D-form") and the single amplicon from the I-allele ("I-form"). Absent from the amplification product of Lin is a third amplicon corresponding to the ACE1-ACE3 product of the I-allele which is required in Appellants claimed method. Thus, Lin does not produce a distinct number of amplicons for each specific genotype. Therefore, the genotype of an individual sample cannot be determined based solely on the number of amplicons produced.

In sum, the fundamental difference between the claimed invention and method of Lin rests on the production of one amplicon (Lin) versus two (Appellants) from the I-allele. This allows Appellants to distinguish each genotype based solely on the number of amplicons produced. By contrast, Lin must positively identify each amplicon to determine which allele it represents.

Lindpaintner does not provide what Lin lacks. As discussed above, Lindpaintner requires the use of two PCR amplifications and four primers in order to determine every ACE genotype. Like Lin, Lindpaintner does not produce a distinct number of amplicons for each genotype. Specifically, Lindpaintner identifies the I/I genotype with one product (first PCR only), the D/D genotype with one product (one product in first PCR, no product in second PCR), and the I/D genotype with two products (either two products in first PCR or one product in each of the first and second PCRs). In no case does Lindpaintner identify the I/D heterozygous genotype using three products as required in Appellants' claimed method.

Thus Lindpaintner, like Lin, does not produce two I-allele amplicons in a single reaction. Accordingly, this strategy does provide Lindpaintner with a different number of amplicons for each ACE genotype.

When viewed together, it is clear that neither Lindpaintner nor Lin provide a method for producing two amplicons from the I-allele of the ACE gene in a single PCR reaction. The production of two I-amplicons is critical to Appellants' claimed method which produces a different number of amplicons for each genotype: one for D/D, two for I/I, and three for I/D. Thus, the prior art cited by the Examiner fails to teach or suggest every element of the claimed invention. For this reason alone, this rejection is traversed and should be withdrawn.

3.4 There is no motivation to combine Lindpaintner and Lin.

A skilled artisan would not be motivated to combine the methods of Lindpaintner and Lin. Lin validates the single-step "real-time" PCR methodology against a "conventional PCR" ACE genotyping method which is substantially identical to the method of Lindpaintner. Specifically, Lin performs parallel genotype determinations using the 2-step, 4-primer method described in Section 2.3.[18] This "conventional PCR" method uses the same primer strategy as that used by Lindpaintner, and achieves the same results. The first PCR uses flanking primers and amplifies a long and a short amplicon representing the I-allele and the D-allele, respectively. A second PCR using an insertion-specific primer and a flanking primer is performed on the samples which did not produce an I-amplicon in order to determine whether its absence is an artifact of preferential amplification of the D-allele or a true representation of the D/D genotype.

When read in its entirety, it is clear that the Lin method is offered as an alternative to the traditional method of Lindpaintner, not as an improvement or a compatible method. Thus, a skilled artisan reading Lin would not combine the real-time PCR method with the traditional ACE genotyping method of Lindpaintner; he would use the real-time PCR method instead of the Lindpaintner method.

3.5 Rejection of claims 40-48, 50 and 68-72.

Claims 40-48 and 50 depend from claim 2, and claims 68-72 depend from claim 67. As discussed above, claims 2 and 67 are unobvious over the combination of Lindpaintner and Lin. Accordingly, all claims that depend from claims 2 and 67 are necessarily also unobvious.

3.6 Summary of the combination of Lindpaintner and Lin.

The prior art cited by the Examiner fails to teach or suggest every limitation of Appellants' claimed method. Appellants' method relies on the production of two amplicons form the ACE I-allele in a single PCR reaction. These two amplicons, in combination with the one amplicon produced from the D-allele permits genotype identification merely by counting the

[18] Lin et al. at p. 662, "2.3. Genotyping of ACE gene I/D allele by conventional PCR".

number of amplicons produced: one amplicon for the D/D genotype, two amplicons for the I/I genotype, and three amplicons for the I/D genotype.

Both Lin and Lindpaintner fails to teach a method for the simultaneous production of two amplicons from the I-allele. The result is that only a single amplicon is produced from the I/I genotype and the D/D genotype. The resulting amplicon must then be further characterized (e.g., by melting curve analysis or amplicon size) in order to determine whether it is representative of the I-allele or the D-allele. Only the heterozygous I/D genotype produces a unique number of amplicons (2) which is fewer than the three amplicons required in Appellants' claimed method.

Furthermore, there is no motivation to combine the teachings of Lindpaintner and Lin. The Lin method was specifically developed as an alternative to the traditional (Lindpaintner) method of ACE genotyping which requires two separate PCR amplifications.

Applicants respectfully submit that claims 2, 40-48, 50 and 67-72 are not obvious over Lindpaintner in view of Lin and request that this rejection be reversed and withdrawn.

4. Rejection of claims 49 and 51-61 under 35 U.S.C. § 103(a) as obvious over Lindpaintner in view of Lin, Soubrier and Buck.

Appellants respectfully traverse the rejection of claims 49 and 51-61 as allegedly being obvious over Lindpaintner in view of Lin, Soubrier et al. (U.S. Patent 5,736,323; "Soubrier"), and Buck et al. (Biotechniques, 27: 528-536, 1999; "Buck").[19] Appellants respectfully request that the rejection be reversed and withdrawn.

Soubrier provides the sequence of Intron 16 (1856 nucleotides) and specifically identifies the 287 nucleotide polymorphic fragment. Soubrier also suggests that primer pairs may be used to amplify all or a portion of Intron 16 for the purpose of determining whether or not the polymorphic fragment is present.

Buck demonstrates the equivalence of sequencing primers. Specifically, Buck performs a sequencing reaction using every primer spaced at a three nucleotide interval over a 300

[19] Final Office Action mailed September 11, 2006 at pp. 11-16.

nucleotide sequence. Virtually every primer used by Buck resulted in a successful sequencing reaction.

The Examiner alleges that, based on the disclosures of Soubrier and Buck, Appellants' specific use of the primers of SEQ ID NOs: 1-3 in the method of Lindpaintner/Lin is *prima facie* obvious.[20] Specifically, the Examiner alleges that every possible primer based on the ACE sequence of Soubrier is obvious because Buck demonstrates that sequencing is successful using a wide variety of primers corresponding to a given sequence.[21] The Examiner erroneously concludes, therefore, that it requires only routine optimization to select the primers of Appellants' SEQ ID NOs: 1-3 based on the Intron 16 sequence of Soubrier which, when combined with the teachings of Lindpaintner and Lin, render claims 49 and 51-61 obvious.[22] Appellants respectfully disagree.

4.1 Soubrier and Buck do not remedy the deficiencies of the combination of Lindpaintner and Lin.

As discussed extensively above, there is no motivation to combine the teachings of Lindpaintner and Lin, and the basic combination fails to teach or suggest every limitation of Appellants' method of claim 2 (the sole independent claim from which claims 49 and 51-61 depend). These deficiencies are not remedied by Soubrier and Buck.

The genotyping strategy suggested by Soubrier is significantly different from Appellants' claimed method and does not remedy the deficiencies of Lindpaintner and Lin. In one embodiment, Soubrier suggest using a single pair of flanking primers which will produce a single amplicon from either the D-allele or the I-allele. The genotype is then determined by "visualiz[ing] the difference in size due to the presence or absence of the insertion."[23] This methodology is substantially the same as the first PCR amplification step of the Lindpaintner method.

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[20] Final Office Action mailed September 11, 2006 at p. 14.

[21] Id. at p. 15.

[22] Id. at pp. 14 and 16.

[23] See col. 3, line 4 through col. 4, line 14; and particularly col. 4, lines 6-14.

In an alternative embodiment, Soubrier suggests using a primer pair consisting of a flanking primer and a primer specific for the fragment (insertion) sequence. This strategy results in a single amplicon when the fragment is present and no amplicon when the fragment is absent.[24] This methodology is substantially the same as the second PCR amplification step of the Lindpaintner method.

Soubrier does not suggest that these two distinct amplifications should be performed sequentially or simultaneously. Even if they were combined, the combination merely results in the method of Lindpaintner. Thus, Soubrier does not significantly expand upon the two-step, four-primer PCR strategy of Lindpaintner as previously asserted by the Examiner.

Specifically, Soubrier fails to teach a method for simultaneously producing two different amplicons from the I-allele using a three-primer system. Furthermore, Soubrier, like Lindpaintner and Lin, fails to suggest simultaneously producing at least three distinct amplicons in a manner that allows for genotype assignment based on the number (not the length) of amplicons.

Buck does not relate to a method for genotype analysis and is not applied by the Examiner for that purpose. Accordingly, Buck does not remedy the failure of Lindpaintner and Lin to teach every limitation of the claimed invention, nor does Buck provide a motivation to combine those teachings.

4.2 Soubrier and Buck do not render obvious the primers of SEQ ID NOs: 1-3.

There is no motivation to combine the teachings of Soubrier and Buck and their combination by the Examiner is improper. Buck is concerned with testing the effectiveness of sequencing primers which are used individually for a DNA polymerase reaction that is randomly terminated prior to the completion of a full copy of the target DNA. By contrast, a PCR reaction creates a full copy of the target DNA and the primers are used in pairs to define the boundaries of the target DNA. Thus, the use of sequencing primers cannot be equated with the use of PCR primers. For this reason alone, the combination of Buck and Soubrier is improper and this ground of rejection is traversed.

[24] Col. 2, lines 58-65.

4.3 Lindpaintner in view of Lin, Soubrier and Buck does not render obvious the Appellants' specific ACE amplicons.

With respect to the rejection of claims 51-52 and 60-61, the Examiner asserts that Lindpaintner's amplicons of 319 bp, 355 bp and 597 bp "approximately" correspond in length to Appellants' amplicons of 123 bp, 157 bp and 410 bp.[25] On its face, this assertion is ridiculous. The two smallest Lindpaintner amplicons are more than twice the size of Appellants'. Furthermore, the specification provides that, when referring to amplicon length, "approximately" encompasses ±10-20%.[26] Insofar as the rejection is applied to claims 60-61, Appellants point out that these claims specify the exact length of each amplicon; no variation in length is encompassed by the claims. Thus, under no reasonable interpretation consistent with Appellants' specification can the amplicons of Lindpaintner render obvious those specified in claims 51-52 and 60-61.

With respect to rejection of claim 53, Appellants point out that the Examiner misstates the limitations of claim 53 and then fails to identify each claimed element with an element in the method of Lindpaintner. The Examiner misstates Appellants' method by asserting that I/D is designated when two amplicons are present (second and third). This is incorrect. Claim 53 specifies ACE genotype assignments as follows: D/D when one amplicon is present (first amplicon), I/I when two amplicons are present (second and third amplicons) and I/D when all three amplicons are present. For clarity, a full comparison of Appellants' and Lindpaintner's genotype assignment criteria follows.

[25] Final Office Action mailed September 11, 2006 at p. 11, ¶¶ 3-4, and at p. 13, ¶¶ 1-2.

[26] Specification at ¶ 30.

Genotype	Appellants' Criteria	Lindpaintner's Criteria
D/D	1 amplicon (123 bp)	1 amplicon (319 bp amplicon in 1st PCR; no amplicon in 2nd PCR)
I/I	2 amplicons (157 bp and 410 bp)	1 amplicon (597 bp amplicon in 1st PCR; 2nd PCR not performed)
I/D	3 amplicons (123 bp, 157 bp and 410 bp)	2 amplicons (319 bp and 597 bp amplicons in 1st PCR; 2nd PCR not performed)
I/D (alternative)		2 amplicons (319 bp amplicon in 1st PCR; 335 bp amplicon in 2nd PCR)

Thus, it is clear that Lindpaintner has different criteria for assigning ACE genotypes compared to that specified in claim 53.

With respect to the rejection of claims 49 and 51-61, Appellants note that these claims depend ultimately from claim 2 which, for the reasons discussed above, is unobvious over Lindpaintner in view of Lin. Accordingly, all claims that depend from claim 2 are necessarily also unobvious.

Applicants respectfully submit that claims 49 and 51-61 are not obvious over Lindpaintner in view of Lin, Soubrier, and Buck, and request that this rejection be reversed and withdrawn.

5. Rejection of claims 62-65 under 35 U.S.C. § 103(a) as obvious over Lindpaintner in view of Lin, and van Bockxmeer.

Appellants respectfully traverse the rejection of claims 62-65 as allegedly being obvious over Lindpaintner in view of Lin and van Bockxmeer et al. (Circulation, 92: 2066-2071, 1995; "van Bockxmeer").[27] The Examiner alleges that Lindpaintner teaches an evaluation of the association of ACE polymorphisms with the risk of ischemic heart disease. The Examiner

[27] Final Office Action mailed September 11, 2006 at pp. 17-19.

further alleges that the teachings of Lindpaintner and Lin render obvious Appellants' claimed method for determining ACE genotype for the previously assertion reasons.[28] Finally, the Examiner alleges that van Bockxmeer correlates ACE genotype with a treatment regimen designed to treat or prevent one or more cardiovascular diseases. Appellants respectfully request that the rejection be reversed and withdrawn.[29]

As discussed extensively above, the basic combination of Lindpaintner and Lin does not render obvious Appellants' claimed method for determining ACE genotype. Specifically, this combination of prior art does not teach or suggest performing a single PCR reaction using three primers (a flanking primer pair and a fragment-specific primer) which produces one, two, or three amplicons, depending upon the ACE genotype. Both Lindpaintner and Lin measure a maximum of two amplicons to make any particular genotype assessment.

The Examiner applies van Bockxmeer merely to demonstrate that the prior art recognized an association between an individual's ACE genotype and their likelihood of developing certain cardiovascular diseases. For this, there is no dispute, but this does not remedy the deficiency of Lindpaintner and Lin. Accordingly, this rejection is traversed and should be withdrawn.

6. Rejection of claim 66 under 35 U.S.C. § 103(a) as obvious over Lindpaintner in view of Lin, van Bockxmeer, Soubrier and Buck.

Appellants respectfully traverse the rejection of claim 66 as allegedly being obvious over Lindpaintner in view of Lin, van Bockxmeer, Soubrier, and Buck.[30] Claim 66 depends from claim 62. As discussed above, the basic combination of Lindpaintner and Lin does not render obvious Appellants' claimed method of determining ACE genotype. Specifically, Lindpaintner and Lin do not render obvious a single PCR reaction using three primers (a flanking primer pair and an insertion-specific primer) which produces one, two, or three amplicons, depending upon the ACE genotype, as claimed by Appellants.

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[28] Final Office Action mailed September 11, 2006 at p. 17, ¶¶ 2-3.

[29] Id. at p. 18, ¶ 4.

[30] Final Office Action mailed September 11, 2006 at pp. 19-22.

Soubrier and Buck do not remedy the deficiency of the combination of Lindpaintner and Lin, nor do Soubrier and Buck teach Appellants' specific primers recited in claim 66. Soubrier merely provides the DNA sequence of ACE Intron 16 and identifies the polymorphic fragment. Buck provides a general teaching that many/most sequencing (not PCR amplification) primers for a given DNA sequence may be successfully used in a sequencing reaction. Thus, there is no motivation to combine the teachings of Buck with those of Soubrier because they are concerned with separate types of polymerase reactions; Soubrier with an amplification reaction and Buck with a sequencing reaction.

Specifically absent from the teachings of Soubrier and Buck is a suggestion to use three primers in an amplification reaction in order to assess the presence or absence of a polymorphic insertion in such a manner that each genotype results in a distinct number of amplicons. Further, neither Soubrier nor Buck specifically teach or suggest using the Appellants' primers of SEQ ID NOs: 1-3.

Finally, van Bockxmeer merely correlates ACE genotypes with certain cardiovascular diseases. Nothing in van Bockxmeer addresses the specific deficiencies of the ACE genotyping methodology that would result from a combination of Lindpaintner and Lin, with or without the further combination of Soubrier and Buck.

Accordingly, Appellants respectfully submit that this rejection is traversed and should be reversed and withdrawn.

7. Rejection of claims 2, 40-48, 50 and 60-70 under 35 U.S.C. § 103(a) as obvious over Teranishi in view of Lin.

Appellants respectfully traverse the rejection of claims 2, 40-48, 50 and 67-70 as allegedly being obvious over Teranishi et al. (J. Hypertension, 17: 351-356, 1999; "Teranishi") in view of Lin.[31] Appellants respectfully request that the rejection be reversed and withdrawn.

Claim 67 encompasses a method for determining the genotype of a gene of interest which has allelic variants based on the presence or absence of an inserted sequence. Claim 2

[31] Final Office Action mailed September 11, 2006 at pp. 7-10.

encompasses a specific variant of the method of claim 67 in which the genotype of ACE is determined. For clarity, the following argument refers specifically to the ACE gene; however, the arguments apply equally to the generic claim 67.

7.1 Teranishi does not remedy the deficiencies of Lin.

Here again, the Examiner obfuscates the issues and analysis. Lin, clearly the closest prior art to Appellants' claimed invention, does not teach or suggest simultaneously producing two amplicons from the I-allele. This deficiency negates the possibility that a different number of amplicons can be produced from each individual ACE genotype.

Teranishi does not remedy that deficiency and is only an incremental change from the method of Lindpaintner, which was previously asserted. Teranishi, like Lindpaintner, performs either one or two separate PCR reactions in order to determine an ACE genotype. The sole difference between the methods of Lindpaintner and Teranishi is that Teranishi reuses one of the flanking primers of the first PCR reaction in the second PCR reaction.[32] Thus, Teranishi can best be described as a two-step, three primer method. Teranishi's output is substantially identical to that of Lindpaintner. Specifically, Teranishi never detects more than two amplicons in order to identify any genotype and Teranishi requires two PCR amplifications to identify all genotypes. Nowhere does Teranishi does not suggest combining the three primers into a single PCR reaction.

7.2 The combination of Teranishi and Lin would not result in Appellants' claimed method.

The Examiner erroneously combines the methodologies of Teranishi and Lin. The proper combination of these methodologies would not result in Appellants' claimed method. Both Lin and Teranishi use a similar primer configuration—a pair of flanking primers and a fragment-specific primer. Lin already uses this primer combination in a single PCR reaction. Furthermore, both Lin and Teranishi produce and detect no more than two amplicon in order to designate any particular genotype. Thus, it is not clear how the method of Teranishi enhances the method of Lin in a manner which is relevant to Appellants' invention.

[32] Teranishi at p. 352, left column, ¶ 3.

7.3 There is no motivation to combine the teachings of Teranishi and Lin.

As discussed extensively above, the method of Lin is presented as an alternative to the traditional ACE genotyping method that requires two separate PCR amplifications. Lin performs a single PCR amplification reaction by producing only two amplicons: a long amplicon from the I-allele and short amplicon from the D-allele. The presence of these two amplicons is detected using a melting curve analysis. Lin validates the new method against a traditional ACE genotyping method which requires two PCR amplifications. Teranishi is only a slight variation on the traditional 2-step 4-primer method which Lin uses as validation. Thus, just as there is no motivation to combine Lin and Lindpaintner, there is also no motivation to combine Lin and Teranishi.

In sum, the combination of Lin and Teranishi, like the combination of Lin and Lindpaintner, fails to teach every limitation of Appellants' claimed invention. Specifically, neither reference teaches a method for the simultaneous production of two amplicons from the I-allele, nor do they teach a method for determining ACE genotype by producing a different number of amplicons from each. Furthermore, there is no motivation to combine the teachings of Teranishi and Lin because the Lin methodology is proposed as an alternative to the "conventional" two-step PCR method of Teranishi. Furthermore, even if the methodologies of Teranishi and Lin were combined, it would not result in Appellants' claimed method. Applicants respectfully submit that claims 2, 40-48, 50 and 67-70 are not obvious over Teranishi in view of Lin and request that this rejection be reversed and withdrawn.

Conclusion

For the reasons discussed above, Appellants respectfully submit that claims 2 and 40-72 are in condition for allowance, and respectfully request that the rejections be reversed and withdrawn, and that the claims be allowed to issue.

Respectfully submitted,

Date: 05/08/2007

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Appendix A: Text of the Claims Involved in the Appeal

1. (Canceled)

2. (Previously Presented) A method of determining an angiotensin converting enzyme (ACE) genotype in a sample, comprising:

amplifying DNA in a single amplification reaction from the sample with a first pair of flanking primers that hybridize to nucleic acid sequences flanking an ACE gene sequence, and a third primer that specifically binds to said ACE gene sequence and together with one of the flanking primers forms a second pair of primers; and

detecting a homozygous ACE genotype by the production of one or two amplification products and a heterozygous ACE genotype by the production of three amplification products.

3-39. (Canceled)

40. (Previously Presented) The method of claim 2 wherein said amplification reaction is by polymerase chain reaction.

41. (Previously Presented) The method of claim 2 wherein the sample is a human sample.

42. (Previously Presented) The method of claim 41 wherein the method distinguishes between genotypes selected from the group consisting of: insertion/insertion, insertion/deletion, deletion/deletion.

43. (Previously Presented) The method of claim 41 wherein the DNA is un-degraded DNA.

44. (Previously Presented) The method of claim 43 wherein the sample is a tissue sample.

45. (Previously Presented) The method of claim 44 wherein the sample is selected from the group consisting of: blood, cultured cells, cells derived from amniotic fluid, and cells derived from chorionic villi.

46. (Previously Presented) The method of claim 2 wherein the sample is blood.

47. (Previously Presented) The method of claim 41 wherein the ACE sequence resides on Intron 16 of chromosome 17q23.

48. (Previously Presented) The method of claim 41 wherein the ACE sequence is a 287 base pair nonsense DNA domain.

49. (Previously Presented) The method of claim 41 wherein the first pair of flanking primers have the nucleic acid sequences 5'-CCA TCC TTT CTC CCA TTT CTC T-3' (SEQ ID NO: 1) and 5'-GGA TGG TCT CGA TCT CCT GA-3' (SEQ ID NO: 2); and the third primer has the nucleic acid sequence 5'-CCT TAG CTC ACC TCT GCT TGT AA-3'(SEQ ID NO: 3).

50. (Previously Presented) The method of claim 41 wherein the DNA sample is from a source selected from the group consisting of: the endothelium of blood vessels, epithelial cells, blood mononuclear cells, macrophages, male germinal cells, and a biological fluid.

51. (Previously Presented) The method of claim 41 wherein the nucleic acid products consist of a first nucleic acid fragment of approximately 123 base pairs, a second nucleic acid fragment of approximately 157 base pairs, and a third nucleic acid fragment of approximately 410 base pairs.

52. (Previously Presented) The method of claim 49 wherein the nucleic acid products consist of a first nucleic acid fragment of 123 base pairs, a second nucleic acid fragment of 157 base pairs, and a third nucleic acid fragment of 410 base pairs.

53. (Previously Presented) The method of claim 52 wherein:

when the first nucleic acid fragment is not present and the second and third nucleic acid fragments are present, the genotype is I/I;

when the first, second, and third nucleic acid fragments are present, the genotype is I/D; and

when the first nucleic acid fragment is present and the second and third nucleic acid fragments are not present, the genotype is D/D.

54. (Previously Presented) The method of claim 2 wherein the first pair of flanking primers have the nucleic acid sequences 5'-CCA TCC TTT CTC CCA TTT CTC T-3' (SEQ ID NO: 1) and 5'-GGA TGG TCT CGA TCT CCT GA-3' (SEQ ID NO: 2); and the third primer has the nucleic acid sequence 5'-CCT TAG CTC ACC TCT GCT TGT AA-3' (SEQ ID NO: 3).

55. (Previously Presented) The method of claim 54 wherein the amplification is by polymerase chain reaction.

56. (Previously Presented) The method of claim 55 wherein the method distinguishes between genotypes selected from the group consisting of: insertion/insertion, insertion/deletion, deletion/deletion.

57. (Previously Presented) The method of claim 55 wherein the ACE sequence resides on Intron 16 of chromosome 17q23.

58. (Previously Presented) The method of claim 55 wherein the ACE sequence is a 287 base pair nonsense DNA domain.

59. (Previously Presented) The method of claim 55 wherein the DNA sample is from a source selected from the group consisting of: the endothelium of blood vessels, epithelial cells, blood mononuclear cells, macrophages, male germinal cells, and a biological fluid.

60. (Previously Presented) The method of claim 55 wherein the nucleic acid products consist of a first nucleic acid fragment of 123 base pairs, a second nucleic acid fragment of 157 base pairs, and a third nucleic acid fragment of 410 base pairs.

61. (Previously Presented) The method of claim 60 wherein:

when the first nucleic acid fragment is not present and the second and third nucleic acid fragments are present, the genotype is I/I;

when the first, second, and third nucleic acid fragments are present, the genotype is I/D; and

when the first nucleic acid fragment is present and the second and third nucleic acid fragments are not present, the genotype is D/D.

62. (Previously Presented) A method for identifying a patient with a heightened risk of suffering from a disease comprising:

determining the angiotensin converting enzyme (ACE) genotype in a sample from the patient by amplifying DNA in a single amplification reaction from the sample with a first pair of flanking primers that hybridize to nucleic acid sequences flanking an ACE gene sequence, the presence of which indicates the presence of a first ACE gene variant, and the absence of which indicates the presence of a second ACE gene variant, and a third primer that specifically binds to said ACE gene sequence and together with one of the flanking primers forms a second pair of primers;

detecting a homozygous ACE genotype by the production of one or two amplification products and a heterozygous ACE genotype by the production of three amplification products; and

correlating the ACE genotype of the patient with a treatment regimen designed to treat or prevent one or more diseases selected from the group consisting of: myocardial infarction, ischemic and idiopathic dilated cardiomyopathy, sudden death in hypertrophic cardiomyopathy, coronary atherosclerosis, and restenosis after percutaneous transluminal coronary angioplasty.

63. (Previously Presented) The method of claim 62 wherein the treatment regimen is designed to treat myocardial infarction or coronary atherosclerosis.

64. (Previously Presented) The method of claim 62 wherein the method distinguishes between genotypes selected from the group consisting of: insertion/insertion, insertion/deletion, deletion/deletion.

65. (Previously Presented) The method of claim 62 wherein the genotype is determined by detecting the presence or absence of each of three nucleic acid products of the amplification reaction.

66. (Previously Presented) The method of claim 63 wherein the pair of flanking primers have the nucleic acid sequences 5'-CCA TCC TTT CTC CCA TTT CTC T-3' (SEQ ID NO: 1) and 5'-GGA TGG TCT CGA TCT CCT GA-3' (SEQ.- ID NO: 2); and the third primer has the nucleic acid sequence 5'-CCT TAG CTC ACC TCT GCT TGT AA-3' (SEQ ID NO: 3).

67. (Previously Presented) A method of determining a genotype for a gene of interest in a sample, comprising:

amplifying DNA in a single amplification reaction from the sample with a first pair of flanking primers that hybridize to nucleic acid sequences flanking a sequence in said gene of interest, the presence of which indicates the presence of a first gene variant, and the absence of which indicates the presence of a second gene variant, and a third primer that specifically binds to said gene sequence and together with one of the flanking primers forms a second pair of primers; and

detecting a homozygous genotype by the production of one or two amplification products and a heterozygous genotype by the production of three amplification products.

68. (Previously Presented) The method of claim 67 wherein said amplification is by polymerase chain reaction.

69. (Previously Presented) The method of claim 67 wherein the sample is a human sample.

70. (Previously Presented) The method of claim 67 wherein the DNA sample is from a source selected from the group consisting of: the endothelium of blood vessels, epithelial cells, blood mononuclear cells, macrophages, male germinal cells, and a biological fluid.

71. (Previously Presented) The method of claim 67 wherein the nucleic acid products consist of a first nucleic acid fragment of approximately 123 base pairs, a second nucleic acid fragment of approximately 157 base pairs, and a third nucleic acid fragment of approximately 410 base pairs.

72. (Previously Presented) The method of claim 67 wherein the nucleic acid products consist of a first nucleic acid fragment of 123 base pairs, a second nucleic acid fragment of 157 base pairs, and a third nucleic acid fragment of 410 base pairs.

Appendix B: Evidence Appendix

None.

Appendix C: Related Appeals and Interferences

None.